ABSTRACT

The present invention provides methods for detecting and identifying sequence variations in a nucleic acid sequence of interest using a detector primer. It has been found that the reduced efficiency of primer extension by DNA polymerases when the 3' end of a primer does not hybridize perfectly with the target can be adapted for use as a means for distinguishing or identifying the nucleotide in the target which is at the site where the diagnostic mismatch between the detector primer and the target occurs. The detector primer hybridizes to the sequence of interest and is extended with polymerase. The efficiency of detector primer extension is detected as an indication of the presence and/or identity of the sequence variation in the target. The inventive methods make use of nucleotide mismatches at or near the 3' end of the detector primer to discriminate between the nucleotide sequence of interest and a second nucleotide sequence which may occur at that same site in the target. The methods are particularly well suited for detecting and identifying single nucleotide differences between a target sequence of interest (e.g., a mutant allele of a gene) and a second nucleic acid sequence (e.g., a wild type allele for the same gene).